Document Number 26

Entry 26 of 38 File: USPT Apr 6, 1993

DOCUMENT-IDENTIFIER: US 5200084 A
TITLE: Apparatus and methods for magnetic separation

DEPR:
It has been discovered, in accordance with the present invention, that the use of polymers, proteins, additives, detergents and like substances, containing a net negative charge, when incorporated into standard cell-biocompatible buffers and used to dilute colloidal magnetic particles, significantly reduces non-specific binding of such colloidal magnetic particles to cells. The non-specific binding of magnetic particles to cells is, in part, a function of the interaction between positively charged sites on magnetic particles and negatively charged sialic acid residues on cell surfaces. Therefore, by adding negatively charged substances to positively charged magnetic particles, a blocking of these positive sites occurs, imparting greater negative surface charge to the particles and, thereby reducing the non-specific binding to cells. As proof of this phenomenon, eight positively charged materials (2 proteins, 6 polymers), when tested separately in the buffer used to dilute colloidal magnetic particles, had extremely adverse effects, significantly increasing the non-specific binding of particles to cells.

Document Number 2

Entry 2 of 38 File: USPT Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981235 A
TITLE: Methods for isolating nucleic acids using alkaline protease

BSPR:
Less tedious methods of isolating nucleic acids are also known. One such method commonly used to isolate and to purify RNA uses magnetic particles, such as paramagnetic particles, to isolate specific species of nucleic acids from a lysate solution containing guanidinium thiocyanate and an anionic detergent. See, for example, PolyATtract.RTM. mRNA Isolation Systems as described in Promega Corporation's 1996 Catalog, pp. 158-160; or see PCT Publication No. WO 96/09308. Another type of nucleic acid isolation method uses silica to isolate plasmid DNA from a bacterial lysate solution containing a guanidinium salt and a base. Boom et al, J. Clinical Microbiol. 28(3): 495-503 (1990). Several silica based resins are commercially available for use in such methods. For example, a specialized silica-based resin, such as one of the Wizard.TM. DNA Purification System resins (commercially available from Promega Corporation, Madison, Wis., U.S.A.) is added to the lysate, and is allowed to bind to the nucleic acid of interest such as plasmid DNA. The resin is then loaded onto a column, washed several times using a vacuum or centrifugal force, and the nucleic acid bound to the resin is then eluted from the column with an elution buffer or water.

(FCA, Pandex) equipped with 365 nm excitation and 450 nm emission filters or five minutes interval and 10.times.gain setting. The increase in fluorescence intensity in a five minutes interval was recorded in arbitrary fluorescence unit (AFU) and presented in Table II.

DEPR:
To the wells of a 96-well microtiter plate were added 20 ul of 0.25% w/v of HIV coated magnetic particles in duplicate. To the wells containing the particles were added 50 ul of positive, borderline and negative specimens diluted 1:100 in specimen dilition buffer (SDB) containing phosphate buffer, protein stabilizers, detergent and antimicrobial agents. After 30 minutes incubation at 37.degree. C., the particles were separated for two minutes on a magnetic separator and washed three times with 100 ul of washed buffer containing salts and detergent. To each well containing particles was added 50 ul of goat antihuman-B-galactosidase (approximately 0.5 ug/ml) conjugate in diluent containing salts, protein stabilizers, glycerol, detergent and antimicrobial agents. After 15 minutes incubation at 37.degree. C., the particles were washed four times as described above. The particles were transferred to the black microtiter plate (Dynatech). To each well containing particles was added 100 ul of a solution containing 4-methylumelliferyl-B-D-galactopyranoside (MUG, Sigma). The plate was incubated at 37.degree. C. and the fluorescence intensity was measured by using a Fluorescence Concentration Analyzer (FCA, Pandex) equipped with 365 nm excitation and 450 nm emission filters at five minutes intervals and 25.times.gain setting. The increase in fluorescence intensity in a five minutes interval was recorded in arbitrary fluorescence unit (AFU) and presented in Table III.

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Document Number 1

Entry 1 of 38 File: USPT Jan 11, 2000

DOCUMENT-IDENTIFIER: US 6013531 A
TITLE: Method to use fluorescent magnetic polymer particles as markers in an immunoassay

DEPR:
To the first two columns of a 96-well microtiter plate was placed 20 ul of 0.25% w/v hepatitis B core antigen (HBcAg) coated magnetic particles prepared as described in Example 34. Sample preparation consisted of various dilutions of a HBcAb positive serum into a negative plasma, followed by a 1:100 dilution of each sample into specimen dilution buffer (SDB). The SDB contained phosphate buffer, protein stabilizers, detergent, and antimicrobial agents. To the wells containing the particles were added 50 ul of each final sample dilution. After 30 minutes incubation at 37.degree. C., the particles were separated for two minutes on a magnetic separator and washed three times with 200 ul wash buffer containing salts and detergent. To each well containing the particles was added 50 ul of goat antihuman IgG-B-D-galactosidase conjugate (0.5 ug/ml) in diluent containing salts, protein stabilizers, glycerol, detergent and antimicrobial agents. After 15 minutes incubation at 37.degree. C. the particles were separated and washed three times as described above and resuspended in 30 ul of IBS. The particles were transferred to the first two columns of a black microtiter plate (Dynatech). To each well containing the particles was added 100 ul of a solution containing 4-methylumbelliferyl-B-galactopyranoside (MUG, Sigma). The plate was incubated at 37.degree. C. and the fluorescence intensity was measured by using a Fluorescence Concentration Analyzer (FCA, Pandex) equipped with 365 nm excitation and 450 nm emission filters at five minutes interval and 10.times.gain setting. The increase in fluorescence intensity in a five minutes interval was recorded in arbitrary fluorescence unit (AFU) and presented in Table I.

DEPR:
To the wells of a black 96-well microtiter plate (Dynatech) were added 20 ul of 0.25% w/v, 3.2 micron, mouse antiHBsAg coated carboxyl magnetic particles in duplicate. To the wells containing the magnetic particles was added 100 ul of neat plasma containing various amounts of HBsAg or a HBsAg-negative plasma. After 30 minutes incubation at 37.degree. C., the particles were separated for two minutes on a magnetic separator and washed once with 100 ul of wash buffer containing salts and detergent. To each will containing the particles was added 20 ul of mouse antiHBsAg--B-galactosidase conjugate in diluent containing salts, protein stabilizers, glycerol, detergent and antimicrobial agents. After 15 minutes incubation at 37.degree. C., the particles were separated and washed five times as described above. To each will containing the particles was added 50 ul of a solution containing 4-methylumbelliferyl-B-D-galactopyranoside (MUG, Sigma). The plate was incubated at 37.degree. C. and the fluorescence intensity was measured by using a Fluorescence Concentration Analyzer

Document Number 5

Entry 5 of 38 File: USPT Mar 2, 1999

DOCUMENT-IDENTIFIER: US 5876924 A
TITLE: Nucleic acid amplification method hybridization signal amplification method (HSAM)

DEPV:
(a) The complex comprising target nucleic acid-probes-beads is then separated from the lysis buffer by means of a magnetic field generated by a magnetic device, which attracts the beads. The magnetic field is used to hold the complex to the walls of the reaction vessel, e.g., a micro-well plate or a microtube, thereby allowing for the lysis buffer and any unbound reactants to be removed, e.g., by decanting, without any appreciable loss of target nucleic acid or hybridized probes. The complex is then washed 2-3 times in the presence of the magnetic field with a buffer that contains a chaotropic agent and detergent in amounts that will not dissociate the complex. A suitable washing buffer for use in the present method comprises about 1.0-1.5M GnSCN, 10 mM EDTA, 100 mM Tris-HCl (pH 8.0) and 0.5% NP-40 (Nonidet P-40, nonionic detergent, Sigma Chemical Co., St. Louis, Mo.). Other nonionic detergents, e.g., Triton X-100, may also be used. The buffer wash removes unbound proteins, nucleic acids and probes that may interfere with subsequent steps. The washed complex may be then washed with a solution of KCl to remove the GnSCN and detergent and to preserve the complex. A suitable concentration of KCl is about 100 to 500 mM KCl. Alternatively, the KCl wash step may be omitted in favor of two washes with ligase buffer.

Document Number 10

Entry 10 of 38 File: USPT May 26, 1998

DOCUMENT-IDENTIFIER: US 5756709 A
TITLE: Compositions for the simultaneous detection and quantification of multiple specific nucleic acid sequences

DEPR:
For the assay, different amounts of either Ngo or Ctr ribosomal RNA, or both, were combined in each tube; the amounts of target varied between 0 and 12.5 fmoles. Final volume of each target nucleic acid dilution was 100 .mu.l; the difference in volume was made up with a solution of 30 mM sodium phosphate (pH 6.8), 3% (w/v) lithium lauryl sulfate, 1 mM EDTA, 1 mM EGTA. The probe reagent was prepared by mixing the 1-Me-AE probe mix and the 1-Me-m-diF-AE probe mix in equal volumes; as in the previous experiment, the probe reagents also contained helper probes. The probe mix contained 190 mM lithium succinate (pH 5.1), 17% (w/v) lithium lauryl sulfate, 3 mM EDTA, 3 mM EGTA and the probes; one hundred microliters of this was added to the target nucleic acid dilutions to yield a final volume of 200 .mu.l. The tubes were shaken to mix and incubated at 60.degree. C. for 90 minutes. The tubes were removed from the water bath and given 1 ml of a solution of 190 mM sodium tetraborate (pH 7.6), 6.89% (w/v) TRITON.RTM. X-102 detergent (polyoxyethylene ether) and 0.01% (w/v) gelatin containing 50 .mu.l of a 1.25% (w/v) suspension of Biomag.TM. 4100 magnetic particles (PerSeptive Biosystems, Cambridge, Mass.) in 0.02% (w/v) sodium azide and 1 mM EDTA. The tubes were incubated further at 60.degree. C. for 10 minutes then removed from the water bath, and the rack was immediately placed on a magnetic separation base and allowed to stand at room temperature for 5 minutes, then the unbound probe was separated from the magnetic bead-bound hybridized probe by decanting the solution. See, Arnold, et al., European Publication No. EPO 281390, which enjoys common ownership with the present invention and which is hereby incorporated by reference herein. The beads and adsorbed hybridized probe were washed once in a solution of 20 mM sodium tetraborate (pH 10.4), 0.1% (w/v) ZWITTERGENT.RTM. 3-14 detergent, then resuspended in 300 .mu.l of 5% (v/v) TRITON.RTM. X-100 detergent.

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